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ABSTRACT

This publication is the third of a series of seven supplementary investigative materials for use in secondary science classes providing up-to-date research-related investigations. This unit is structured for grade levels 7 through 12. It is concerned with the role of carbohydrates as important nutrients for consumers. This guide will enable students to study and identify certain carbohydrates. The first part of this guide provides the teacher with: (1) materials needed; (2) background information; (3) preparing reagents; and (4) suggested reading and films. The second part provides students with background information and two investigations: (1) testing for unknown carbohydrates, and (2) determination of stored carbohydrates in vegetables. Each investigation consists of: (1) materials needed for a four-student team; (2) procedures; (3) questions for thought; (4) extending the investigation; and (5) suggested readings. (HM)

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science study aids

carbohydrates: food energy source

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SE 024 379

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carbohydrates:
nature's energy source

Developed by Bill McConnell, a science teacher at Redwood High School in Larkspur, California. Mr. McConnell prepared the manuscript in cooperation with Agricultural Research Service (ARS) scientists, U. S. Department of Agriculture, at the Western Regional Research Center, Berkeley, California.

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TO THE TEACHER

This Science Study Aid (SSA) is based upon work of the U. S. Department of Agriculture's Agricultural Research Service (ARS) conducted at the Western Regional Research Center in Berkeley, California. It is concerned with the role of carbohydrates as important nutrients for America's consumers. A continuing program of research by ARS scientists is under way to evaluate data on the composition and nutritive value of foods in all the various forms in which they are eaten. Tables of food composition constantly are being revised to keep up with changing processing methods and improved measuring techniques. Food

MATERIALS LISTS

For your convenience, we have listed below the materials needed to perform the Investigations in this Science Study Aid. The following list gives the quantities needed for each team of four students.

STUDENT MATERIALS

12 15 x 150 mm test tubes
1 test tube rack
1 test tube holder
1 800 ml beaker
1 4-inch ring clamp
1 6-inch ring clamp
1 support stand
1 Bunsen burner
1 10 ml graduated cylinder
1 wire gauze screen
1 spot plate
6 25 x 200 ml test tubes
3 glass stirring rods
4 15 ml centrifuge tubes
1 centrifuge (per class)
1 household blender (per class)
1 triple-beam balance
200 ml 80% ethyl alcohol
2 liters distilled water
60 ml 50% perchloric acid
6 250 ml flasks
1 1000 ml beaker
6 150 ml beakers
1 light source (e.g., gooseneck lamp)
1 funnel
3 pieces filter paper
3 300 ml flasks
30 ml Anthrone-sulfuric reagent
3 1/2-pound quantities of fresh peas purchased at 3 different produce markets, OR 3 different brands of frozen peas in 12-oz. pkgs.
ice and tap water

TEACHER MATERIALS

Unknown carbohydrate solutions in numbered 30 ml dropping bottles:

Distilled H_2O

1% glucose solution
1% fructose solution
1% sucrose solution
1% starch solution
Reagents: (for class of 28 working in pairs)
500 ml ea of:
Benedict's solution
Lugol's solution
Selivanoff's solution
Barfoed's solution
1% fructose solution
1% glucose solution
0.1% xylose solution
1% sucrose solution
1% mannose solution
distilled water
Reagents: Bial's solution
Phenylhydrazine reagent
Tes-tape or Clinistix paper

composition tables are indispensable to dietitians, teachers, and the medical profession. Of special interest is the nutrition data on carbohydrates and their relation to human nutritional needs.

This SSA will enable students to study and identify certain carbohydrates, and perform research techniques developed by ARS scientists at the WRRC. Students will conduct their studies of carbohydrates in much the same way that scientists do in their research programs.

This SSA uses the inquiry method to help develop meaningful group or class discussions. Most activities can be carried out using standard laboratory equipment. Also, for students who show special interest and for the more able learner, extensions of the investigation are provided.

The material will be of particular value in chemistry, physiology, and advanced biology courses during studies of biochemistry, energy, cycles, food chemistry, and nutrition. An essential part of this SSA is the background material. Students should receive the background information a few days prior to the laboratory investigations. This will allow sufficient time for you and your students to survey the suggested reading materials at the end of the SSA, along with teacher-assigned readings from textbooks. Students may work in pairs or in teams to carry out the activities.

BACKGROUND: CARBOHYDRATES

As this continuing research on carbohydrates proves successful, better crops and food processing techniques can be developed to assure the consumer of better health and a more nutritious diet.

Carbohydrates, as their name implies, are a group of organic compounds containing the elements carbon, hydrogen, and oxygen generally in the ratio of about 1:2:1. Most monosaccharides have a basic sugar unit with 6 carbon atoms, while disaccharides and polysaccharides have 6 carbon sugar units linked together by various oxygen containing bonds. On the basis of the number of these 6 carbon compounds (sugar units) that are bonded together to make longer units, the carbohydrates are roughly divided into three classes: monosaccharides (one sugar) which consists of a single 6 carbon molecule (for example, fructose); disaccharides (two sugars) which consist of 2 single sugar molecules linked together (table sugar or sucrose is made up of glucose and fructose); and polysaccharides (many sugars) which consist of 3 or more sugar molecules linked together (plant and animal starch are long chains of glucose units). Starch and glycogen are storage forms of carbohydrates; starch is usually produced in plants and glycogen in animals and humans. They are insoluble in water and thus remain in cells until needed as sources of energy for life processes.

In your first research Investigations, you will find that carbohydrates can be identified by color reactions with specific reagents. These tests can be used quantitatively as well as qualitatively by measuring the variation in color obtained when using different concentrations of reagent. Your final Investigation in this SSA will provide an opportunity for you to perform tests for carbohydrates.

PREPARING REAGENTS

All carbohydrate solutions should be in numbered 30 ml dropping bottles. Do not identify any of the prepared carbohydrate solutions. Let the students rely on their knowledge of the tests used to identify each solution. (CAUTION: If students mix dropping bottle pipettes, all work will be meaningless.)

You may purchase the prepared reagents from supply houses or prepare your own as follows: (500 ml for class of 28)

1. Barfoed's solution

   33 g copper acetate
   4 ml glacial acetic acid
   500 ml distilled water
   (to distinguish between monosaccharides and disaccharides)

2. Benedict's solution

   8.6 g copper sulfate
   86.5 g sodium citrate
   50.0 g sodium carbonate
   500 ml distilled water
   (to detect reducing sugars)

3. Bial's solution

   1.5 g Orcinol
   500 ml concentrated hydrochloric acid
   1 ml 10% ferric chloride solution
   (to detect pentose (5C) sugars)

4. Selivanoff's solution

   0.25 g Resorcinol
   500 ml dilute (1:2) hydrochloric acid
   (to distinguish between ketose and aldose sugars)

5. Lugol's solution

   25 g iodine
   50 g potassium iodide
   500 ml distilled water
   (to detect certain plant starches)

6. Phenylhydrazine Reagent

   Mix in a mortar equal weights of phenylhydrazine hydrochloride and sodium acetate to bring the dry volume to approximately 100 cubic centimeters. (To detect mannose).

7. Glucose test paper (to test for glucose)

   Can be purchased in any drug store.

8. Anthrone-Sulfuric Acid Reagent

   Dissolve 1 gram of anthrone in 500 ml of cold 95% sulfuric acid. Store in refrigerator until used.

9. Iodine-Potassium Iodide Reagent

   1 gram Iodine
   10 grams Potassium Iodide
   500 ml distilled water

10. Perchloric Acid Reagent, 50%*

    350 ml 70% Perchloric Acid
    150 ml distilled water

* Store in glass-stoppered containers ONLY. If you are not familiar with this reagent, we suggest that you read the following reference:

Manufacturing Chemists Association Safety Committee, 1954. Guide for Safety in the Chemistry Laboratory, D. Van Nostrand Company, Inc., New York. Pages 117-120.

SUGGESTED READING FOR THE TEACHER

Laboratory Studies in Biology-668: Carbohydrate Chemistry, Abramoff, Peter and R. G. Thomson, 1966, W. H. Freeman and Co., San Francisco and London.

Practical Physiological Chemistry, Hawk, P. B., B. L. Oser, and W. H. Summerson, 1954, (13th ed.), McGraw-Hill, New York.

Cellular Physiology and Biochemistry, McElroy, W. D., 1964 (2nd ed.), Prentice-Hall, Englewood Cliff, N. J.

The Carbohydrates: Chemistry, Biochemistry, Physiology, Pigman, W. W., 1957, Academic Press, New York.

Carbohydrates of Living Tissue, Stacey, M., and S. A. Barker, 1962, Van Nostrand, Princeton, N. J.

FILMS

Cell Biology, Part I: From Atoms to Organisms, No. 12, 1960, (sound, color, 27 min.), purchase or rent, AIBS (McGraw-Hill).

Cell Biology, Part I: Patterns of Energy Transfer, No. 6, 1960, (sound, color, 30 min.), purchase or rent, AIBS (McGraw-Hill).

Multicellular Plants, Part III: Role of the Green Plants, No. 5, 1961, (sound, color, 26 min.), purchase or rent, AIBS (McGraw-Hill).

TABLE 1

QUALITATIVE CARBOHYDRATE TESTS

	Carbohydrate Tests	Reagent	Positive Reaction
1.	For reducing sugars, e.g., glucose, fructose	Benedict's Solution	Color change when heated: blue → green → orange → brown; color reaction depends on concentration of reducing sugar.
2.	To distinguish between monosaccharides and disaccharides, e.g., glucose, sucrose	Barfoed's Solution	A positive test for monosaccharides is the appearance of a red precipitate after heating for 1-2 minutes. Disaccharides take much longer (15-30 min.) to form ppt.
3.	For Pentose (5C) sugars, e.g., xylose	Bial's Solution	A positive test for pentoses results when, upon heating for 25 minutes, a color change from yellow to dark green occurs.
4.	For aldose and ketose sugars, e.g., glucose and fructose	Selivanoff's Solution	A positive test for ketose sugars is the appearance of a red color after heating for ½ - 1 minute. Aldose sugars will react with Selivanoff's solution; however, they require longer heating.
5.	For the monosaccharide, mannose	Phenylhydrazine Reagent	With this reagent, mannose forms a white crystalline precipitate in 10-15 min. at room temperature.
6.	For certain plant and animal starches	Lugol's Solution	Color change: amber (yellow) → blue → blue-black -- color reaction depends on concentration of starch.
7.	For glucose	Glucose test paper	Color change: red to blue, or yellow to green.

TO THE STUDENT

Do you know that, on planet Earth, the only thing that most living cells can use directly for an energy source is sugar? Carbohydrates (sugars), along with many other important compounds such as enzymes, fats, and nucleic acids, enable the living cell to carry out the important business of life. Would you like to learn more about these biologically important organic compounds known as carbohydrates? After all, they are the direct source of energy for most living things on this planet.

Agricultural Research Service (ARS) scientists have long been interested in the role of carbohydrates in human nutrition. New and exciting things are being discovered by ARS scientists as they carry out a continuing program of research, evaluating data on the composition and nutritive value of foods for American consumers. Of special interest to ARS is the nutrition data on carbohydrates and the amount of these important organic compounds found in our food. A technique to simplify the collection of these data was discovered by Dr. R. M. McCready and his colleagues at the Western Regional Research Center, Berkeley, California. The problem which led to the discovery of this analytic technique was twofold.

First, growers, food processors, canners, and agricultural scientists were interested in a more accurate way to determine the maturity of fruits and vegetables for harvest and processing. Many of the older methods used to determine maturity were unreliable; for example, the size (diameter) was used to determine the levels of maturity in peas. However, there was nothing to refute the contention that a No. 4 size pea in one pod might be as mature as a No. 6 size pea in a different pod. Scientists knew that the amount of stored carbohydrate in fruits and vegetables was the real index for determining maturity and that a new chemical test was needed. Dr. McCready and his co-workers developed new procedures that proved to be more accurate than other methods and allowed scientists to better correlate maturity with a chemical property in plant material.

For years, the analytic methods used to detect carbohydrate compounds in fruits and vegetables were time-consuming and often inaccurate. In addition, these older chemical tests required large amounts of plant material. A more expeditious method for carbohydrate analysis in fruits and vegetables, applicable to small amounts of material, was required. Dr. McCready's technique saved hours of the working scientists' time and, with modification, analysts now can make accurate tests using only one sample, thus solving the second part of the problem.

In this SSA, to demonstrate some of the ways in which carbohydrates can be identified, you will carry out various tests like ARS scientists. The tests will serve to detect and differentiate certain carbohydrates. Studying the reactions involved in these tests will serve as a review of some important aspects of carbohydrate chemistry.

Finally, a successful research program always uncovers new questions which lead to new assumptions along with ideas to test these assumptions. The careful and informed worker will always generate more questions than his present investigative activities can answer. How about you? Will your work lead to new and more interesting ideas related to the study of carbohydrates?

INVESTIGATION 1: Part A,
Testing for Unknown Carbohydrates

In this part of Investigation 1, you will test unknown solutions for some of the carbohydrates mentioned in the section of this SSA entitled "To the Student". As you progress through the Investigation, see if you can identify the carbohydrates that are provi-

ded by your teacher. Design a data table similar to the one in Figure 2. Record your data in your notebook. When you have finished your laboratory work, study the data that you have recorded and consider this information as you answer the questions at the end of this Investigation.

MATERIALS: (for each pair or team on first day)

5 15 x 150 mm test tubes
1 test tube rack
1 test tube holder
1 test tube brush
1 800 ml beaker
1 4" support ring
1 6" support ring
1 support stand
1 Bunsen burner
1 10 ml graduated cylinder
1 wire gauze screen
Unknown carbohydrate solutions in numbered 30 ml dropping bottles:
- Distilled H_2O
- 1% glucose solution
- 1% fructose solution
- 1% sucrose solution
- 1% starch solution

Reagents: (for class of 28 working in pairs)
- 500 ml each of:
- Benedict's solution
- Lugol's solution
- Selivanoff's solution
- Barfoed's solution

PRELIMINARY PROCEDURE (First day)

1. Fill the 800 ml beaker with water until it is just a little more than half full (500 ml water). Prepare the water bath as shown in Figure 1 and bring the water to a boil.
2. Be sure that all glassware is clean before proceeding with the tests. Dirty glassware will result in inaccurate data.
3. When the test tubes are labeled and prepared for heating in the water bath, place all 5 tubes in the water bath at the same time! Do not vary from the suggested times for each test.

PROCEDURE (First day)

1. Label the test tubes 1 through 5 and place them in the test tube rack. Put 5 ml of Benedict's solution in the graduated cylinder. Transfer this reagent to tube number 1, then fill the remaining tubes with Benedict's solution to the level of the first test tube. Check to see that the volumes are approximately the same in each tube. For each carbohydrate to be tested, put 0.5 ml (10 drops) of the carbohydrate solution in the tube that matches the correct number on the dropping bottle. Into test tube number 5, put 0.5 ml (10 drops) distilled water. What is the purpose of preparing this tube? Mix the solutions well. At the same time, place all five tubes in the boiling water bath for three minutes. After the three-minute boiling period, carefully remove the tubes from the water bath using a test tube holder and place them in the test tube rack. Record the color for each carbohydrate tested and indicate whether or not the test was positive or negative. Benedict's solution contains compounds that are reduced to cuprous oxide (Cu_2O) which forms a precipitate when heated with reducing sugars such as glucose and fructose. Which tubes contained reducing sugars?
2. After recording the data, thoroughly wash the test tubes using a test tube brush. Rinse each tube with a small amount of distilled water and return them to the test tube rack. You are now ready for the second test.
3. Repeat the first step of the procedure (paragraph 1 above) ONLY THIS TIME add 5 ml of Barfoed's solution instead of Benedict's to each tube. Add the carbohydrates as directed in the first step and, at the same time, place all tubes in the boiling water bath for 2 minutes. Do not allow

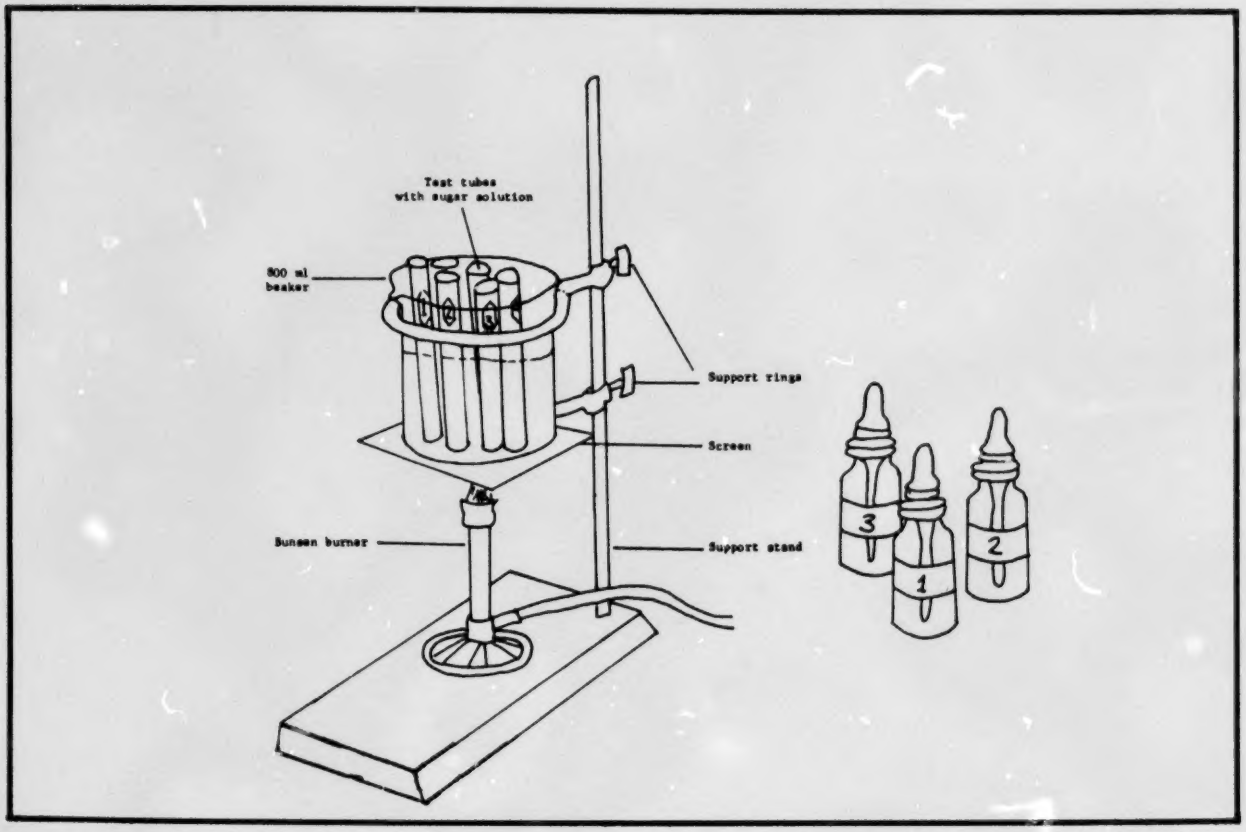

FIG. 1

them to heat longer! Remove the tubes and record the results, noting the time of the appearance of the red precipitate. Barfoed's test is used to distinguish between monosaccharides (glucose, fructose) and disaccharides (sucrose, maltose). In this test, disaccharides will not reduce Cu^{++} to Cu_2O, whereas monosaccharides will reduce Cu^{++} when heated for 2 minutes. Identify the monosaccharides and suggest which carbohydrates they might be. Which tubes contain disaccharides or polysaccharides?

4. The third test will again follow the procedure outlined in the previous steps except that, this time, fill tubes with 5 ml of Selivanoff's solution. When the carbohydrates have been added, place the tubes, at the same time, into the boiling water bath for 4 minutes. Watch the tubes carefully and record the time at which any color changes occur. The Selivanoff's test is used to differentiate between ketose sugars (fructose) and aldose sugars (glucose). Ketose sugars react much more rapidly with this reagent than do aldose sugars. Ketoses give a red color in 1/2 to 1 minute, but aldoses require much longer heating for color production. Record the results of this test in your data table, noting the time of appearance of a red color.

5. For the final test, put 3 ml of distilled water in each of the clean test tubes. Add the carbohydrates and mix the solutions well. Put 10 drops (0.5 ml) of Lugol's solution into each tube. A positive reaction for starch (polysaccharide) is a color change from amber to dark blue. Which tube contains starch? (Note: you do not heat the tubes for this test.) Record the results in your data table.

6. Return all equipment to its proper place and clean your work area. Then, consider the information in your data table and answer the following questions:

QUESTIONS FOR THOUGHT
(Investigation 1, Part A)

1. In step 1, which numbered solution gave a positive test for reducing sugars? How do you know these carbohydrates are reducing sugars? Explain.

2. Describe the color changes that occur when testing a reducing sugar with Benedict's solution: do you think the reaction might tell you anything about the concentration of carbohydrates? Explain.

3. Inasmuch as you know which sugars were being tested, how can you identify the monosaccharides from the disaccharides? What sugars give a positive test with Barfoed's solution within 2 minutes?

4. How can you distinguish between fructose and glucose using the tests outlined in this investigation? Which numbered solution is fructose? Which is glucose? Explain.

5. How can you distinguish starch from the other carbohydrates? Explain.

6. Consider the following questions:

   a. What constitutes a positive test for reducing sugars?
   b. What constitutes a positive test for fructose (ketose sugar) and glucose (aldose sugar)?
   c. How can you distinguish between starch and glucose? Explain your answer.

7. If you were an ARS scientist, how might these tests help you to identify the carbohydrate content of certain foods?

INVESTIGATION 1: Part B,
Testing for Unknown Carbohydrates

Again,in this part of Investigation 1, you will test unknown solutions for carbohydrates. As you progress through the Investigation, try to identify the carbohydrates provided by your teacher. Record your data in your notebook, and when you have finished the laboratory work, study the data that you have recorded and then consider the questions at the end of this Investigation. Figure 3 suggests how you might design your data table.

MATERIALS: (for each pair or team on second day)

12 15 x 150 ml test tubes
1 test tube rack
1 test tube holder
1 800 ml beaker
1 4" support ring
1 6" support ring
1 support stand
1 Bunsen burner
1 10 ml graduated cylinder
1 wire gauze screen
1 spot plate
Unknown carbohydrate solutions in numbered 30 ml dropping bottles:
   1% fructose solution
   1% glucose solution
   0.1% xylose solution
   1% sucrose solution
   1% mannose solution
   Distilled Water
Reagents: Bial's solution
Phenylhydrazine reagent
Tes-tape or Clinistix paper

PRELIMINARY PROCEDURE (Second day)

1. Fill the 800 ml beaker with water until it is just a little more than half full (500 ml

water). Prepare the water bath as shown in Figure 1 and bring the water to a boil.

2. Be sure that all glassware is clean before proceeding with your tests. Dirty glassware will result in inaccurate data.

PROCEDURE (Second day)

1. Label the test tubes 1 through 6 and place them in the test tube rack. Pour 3 ml of Bial's solution into the graduated cylinder. (CAUTION: Bial's solution is a reagent made with concentrated hydrochloric acid. Do not spill on skin or clothing.) Put this reagent in test tube 1, then fill the others with an equal volume of the reagent. For each carbohydrate to be tested, put 0.5 ml (10 drops) of the carbohydrate solution in the tube that matches the correct number on the dropping bottle. Into test tube number 6, put 0.5 ml (10 drops) distilled water. At the same time, place all six tubes in the boiling water bath for 25 minutes. Bial's test is used to identify pentose sugars (monosaccharides with 5 carbon atoms), and a positive test for the pentoses will give a green color. However, other carbohydrates may give a brown color with this test! As the tubes are heating, go on to steps 2 and 3. However, when the test is finished, be sure to record your results.

2. Label the test tubes 1 through 6 and place them in your test tube rack. Put 1/4 inch of dry solid phenylhydrazine reagent into each clean test tube. (CAUTION: Poisonous, do not spill or get on skin.) To each test tube, add 2.0 ml (40 drops) of carbohydrate solution as directed in step 1. Add distilled water to the 6th tube. Mix until the reagent has dissolved. Allow reagent to stand for 10-15 minutes. The formation of a white crystalline precipitate indicates the sugar mannose. Record your results in your data table.

3. Put 5 drops of each of the carbohydrates to be tested in the recesses of a white procelain spot plate. You may wish to number each recess to correspond to the carbohydrate being tested. Dip a dry piece of glucose test paper into each carbohydrate and observe any change in color. A positive test for reducing sugars is indicated by a color change. Record the results in your data table. When finished with your tests, return all equipment and materials to their proper place and clean your work area. Then consider the questions that follow.

QUESTIONS FOR THOUGHT
(Investigation 1, Part B)

1. Which numbered solution contains 5 carbon atoms? How do you know? What test was used to establish this fact? How did you know the test was positive? Explain.

2. Which solution contained mannose? How do you know? Describe the positive test reaction for the carbohydrate mannose.

3. Glucose test papers are used by diabetics to test for the presence of certain sugars in urine samples. Which carbohydrates give a negative test for urine sugar? Which sugars give a positive test? How might positive tests help the diabetic to adjust insulin intake? Explain.

4. How might ARS scientists determine the concentration of pentoses in select grains? Explain. Which test in this investigation would they most likely use? Why?

EXTENDING THE INVESTIGATION

1. Design an experiment using the tests in these investigations to determine the carbohydrates in select foods.

2. The quantity of reducing sugar in a solution is a function of the amount of precipitate formed during the Benedict's test. Design an experiment to determine quantitatively the reducing sugar content of select solutions and foods. How might you develop standard solutions for this experiment?

3. Design an experiment, based on the tests for carbohydrates in this investigation, that will determine the intake of sugars by students in your class after eating such foods as fruit, cookies, grains, and soft drinks.

4. Test some edible wild plants for carbohydrate content and suggest why some plants, rather then others, would be more suitable for an energy source to mountain climbers, backpackers, and people visiting the nation's wilderness areas.

INVESTIGATION 2: DETERMINATION OF STORED CARBOHYDRATES IN VEGETABLES

Now that you have performed tests for unknown carbohydrates, you will work with an analytic technique developed by scientists at the Western Regional Research Center, Berkeley, California. In this Investigation, you will determine the maturity of vegetables by analyzing them for stored carbohydrates. Be sure to record the results of your tests in a data table.

READ AND STUDY THE PROCEDURES BEFORE YOU BEGIN THE INVESTIGATION.

MATERIALS (for team of 4 students)

6 25 x 200 mm test tubes
3 glass stirring rods
4 15 ml centrifuge tubes
1 centrifuge
1 household blender
1 triple-beam balance
1 300 ml flask
6 250 ml flasks
1 water bath (as in Investigation 1)
1 light source
3 ½ pound quantities of fresh peas purchased at 3 different produce markets - OR - 3 different brands of frozen peas in 12-oz. packages
200 ml 80% ethyl alcohol
2 liters distilled water

60 ml 50% perchloric acid*
1 funnel
1 filter paper
30 ml Anthrone-sulfuric acid reagent
1 1000 ml beaker
3 150 ml beakers
1 25 ml graduate cylinder
ice and tap water

*YOUR TEACHER MAY WISH TO ADD THIS REAGENT TO YOUR TUBES IN STEP 3 SO MEMBERS OF YOUR TEAM WILL NOT HAVE TO HANDLE IT!

PRELIMINARY PROCEDURE

1. Prepare a water bath as you did in Investigation 1. Only this time, add 300 ml of tap water to your beaker. Bring the water to a boil.

2. Put 200 ml of 80% ethyl alcohol in a 300 ml flask and heat in your water bath until the alcohol is hot. Turn off the heat source and leave the flask of alcohol in the water bath until you are ready to use it.

3. Be sure all glassware is clean! Dirty glassware will result in inaccurate data. Rinse with distilled water only!

PROCEDURE

1. Blend 100 grams of fresh or frozen peas separately, from each of the three samples, with an equal weight of distilled water in a household blender for 5 minutes. Be sure the blender jar is washed and rinsed with distilled water before working your second and third sample! Then, treat each sample separately as outlined in the steps that follow in this procedure.

2. Weigh a 5 gram sample of the pea slurry in a 100 ml beaker. Divide the sample equally into four 15 ml centrifuge tubes. Fill each tube with hot 80% alcohol and stir. Centrifuge all four tubes until the upper layer of alcohol in each centrifuge tube is clear. Pour off this upper layer being careful not to pour off any of the starch residue.

Repeat the above procedure using hot 80% ethyl alcohol three times. Rinse the residue from each centrifuge tube into a 25 x 200 mm test tube using distilled water. Bring the volume in the tube to 10 ml.

3. Cool the test tube and contents in an ice water bath and stir while adding 13 ml of perchloric acid reagent. Stir occasionally for 15 minutes.

4. After 15 minutes, add 20 ml of distilled water while stirring the solution in the tube. Rinse this solution into a 250 ml flask and bring the total volume to 100 ml with distilled water. Filter the solution into a 150 ml flask and label sample number 1. Prepare sample number 2 and 3 in exactly the same way, following the procedures in steps 2, 3, and 4.

5. Dilute 5 ml of sample #1 in 250 ml of distilled water. Pour 5 ml of the dilute carbohydrate solution into a clean 25 x 200 mm test tube. Cool the tubes in an ice water bath for a few minutes and then add 10 ml of Anthrone-sulfuric acid reagent to the tube. Thoroughly mix the solution using a clean glass stirring rod. At the same time, heat the water to boiling in your water bath. Then, prepare samples #2 and #3 in exactly the same way. When all 3 samples are prepared, place the three tubes in the boiling water bath for 7.5 minutes. Remove and cool rapidly in cold water until the contents of the tubes are approximately 25° C.

6. When 3 tubes have cooled, hold them in front of a light source and determine the color intensities of the samples. The more intense the color, the more stored carbohydrate in the sample. When you have made your color determinations, record this data and consider the "Questions for Thought".

QUESTIONS FOR THOUGHT

1. Discuss the relationship between maturity of plant material and the "color intensities" of the Anthrone-sulfuric acid test.
2. Which pea sample had the greatest amount of stored carbohydrate? Which had the least?
3. Did the size (diameter) of peas from each sample vary? If they did, relate this to the results of your test.

EXTENDING THE INVESTIGATION

1. Determine the maturity of a number of vegetables, other than peas, using the procedures in Investigation 2.
2. If you live near the coast, try the procedures in Investigation 1 on select marine algae.

SUGGESTED READING FOR STUDENTS

The Chemicals of Life, Asimov, Issac, 1954, Abelard-Schuman, New York.

Introduction to Comparative Biochemistry, Baldwin, Ernest, 1949, Cambridge University Press, New York.

The Chemistry of Living Cells, Downes, H. R., 1962, Harper & Row, Pub., New York, Chap. 15.

A Direct Entry to Organic Chemistry, Read, John, 1959, Harper & Row, Pub., New York.

Science for Living: A Report on Progress from Agriculture Research, U. S. Department of Agriculture, 1974, Bulletin No. 363, Washington, D. C.

science study aids

are a series of supplementary investigative materials for use in secondary science classes, grades 7 - 12. The materials are based on federal and private research programs. They are written by secondary science teachers working with scientists at research facilities throughout the country. Before being published, they are tested in the laboratory and in classrooms of cooperating teachers.

Several times during the year, new SSA's are developed. If you want to be notified of their availability, request that your name be added to Wordwork's mailing list. Because we cannot provide enough copies for students, we have designed SSA's so that teachers can easily reproduce the student portion for their classes.

We hope that you find the Science Study Aid Series a valuable supplement to your science curriculum. We welcome you comments on the SSA's that you receive.

Science Study Aids
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